

Attorney Docket No. P64765US1
Serial No. 09/779,461

- c) determining from said intermediate statistical data a distribution of particles as a function of at least two arguments, wherein one argument is a specific brightness of the particles, or a measure thereof, and another argument is a diffusion coefficient of the particles, or a measure thereof,

wherein said distribution function of particles is determined by fitting the experimentally determined probability functions $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$ by corresponding theoretical probability functions $P_1(n_1), P_2(n_2), \dots$

and

wherein said theoretical probability distributions $P_1(n_1), P_2(n_2), \dots$ are calculated as functions of apparent concentrations and apparent brightness which depend on the widths of the counting time intervals in the different sets.

39. The method according to claim 38 wherein, in calculations of the theoretical distributions $P_1(n_1), P_2(n_2), \dots$, an optical spatial brightness function $B(x)$ is accounted for by a separately determined relationship between brightness B and volume elements dV .
40. The method according to claim 39, wherein the relationship between the spatial brightness B and volume elements dV is expressed through a variable $x = \ln(B_0/B)$ by a relationship

$$\frac{dV}{dx} = A_0(1 + a_1x + a_2x^2)x^{a_3},$$
 where B_0 is maximum brightness and A_0, a_1, a_2 and a_3 are empirical parameters of the spatial brightness function.

41. The method according to claim 38 wherein each set of counting time intervals consists of intervals of equal width while different probability functions $\hat{P}_{T_1}(n_1), \hat{P}_{T_2}(n_2), \dots$ correspond to counting time intervals of different widths T_1, T_2, \dots .
42. The method according to claim 42, wherein the apparent concentration is calculated as

Attorney Docket No. P64765US1
Serial No. 09/779,461

$$c_{app}(T) = \frac{c_{app}(0)}{\Gamma(T)},$$

the apparent brightness is calculated as

$$q_{app}(T) = q_{app}(0)\Gamma(T),$$

and $\Gamma(T)$ is calculated as

$$\Gamma(T) = \frac{1}{c_{app}(0)q_{app}^2(0)T^2} \int_0^T dt_1 \int_0^T dt_2 G(t_2 - t_1),$$

where $G(t)$ denotes autocorrelation function of fluorescence intensity and T denotes the width of the counting time interval.

43. The method according to claim 38 wherein the counting time intervals in each set are consecutive in time.
44. The method according to claim 38 wherein, counting time intervals in each set overlap.
45. The method according to claim 38 wherein said intermediate statistical data are processed applying inverse transformation with regularization and/or constraints.
46. The method according to claim 38 wherein the theoretical distributions $P_1(n_1), P_2(n_2), \dots$ are

calculated through their generating functions $G_{P(n)}(\bar{\xi}) = \sum_n \bar{\xi}^n P(n)$.

47. The method according to claim 38 wherein said distribution function of particles is determined by fitting the experimentally determined probability functions $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$ by corresponding theoretical probability functions $P_1(n_1), P_2(n_2), \dots$.

Attorney Docket No. P64765US1
Serial No. 09/779,461

48. The method according to claim 47, wherein the theoretical probability functions $P_1(n_1), P_2(n_2), \dots$ are calculated through their generating functions $G_{P(n)}(\xi) = \sum_i \xi^n P(n)$.
49. The method according to claim 46, wherein the generating function is calculated using the expression $G(\xi) = \exp[\int dq c(q) \int d^3r (e^{(\xi-1)qTB(r)} - 1)]$, where $c(q)$ is the density of particles with specific brightness q , T is the length of the counting time interval, and $B(r)$ is the spatial brightness profile as a function of coordinates.
50. The method according to claim 47, wherein said generating functions are calculated using the formula $G_{P(n)}(\xi) = \exp[\sum_i c_i \int (e^{(\xi-1)q_i B(r)T} - 1) dV]$ in which c is an apparent concentration and q is an apparent brightness which both depend on the width of the counting time interval T .
51. The method according to claim 38 wherein concentrations of particles are selected to be approximately one or less molecules per measurement volume.
52. The method according to claim 38 wherein said photon detector is either an avalanche photodiode or a photomultiplier.
53. The method according to claim 38 wherein at least two photon detectors are used monitoring fluorescence of different wavelengths or polarization.
54. The method according to claim 38 wherein said fluorescent particles are characterized by applying an homogeneous fluorescence assay.
55. Use of a confocal apparatus for performing the method according to claim 38.
56. Use of a confocal apparatus for performing the method according to claim 38, said confocal apparatus comprising:
- a radiation source (12) for providing excitation radiation (14),
 - an objective (22) for focusing the excitation radiation (14) into a measurement volume (26),